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The question concerning the source of nematodes in finished water has been answered by clearly demonstrating that these invertebrates do not breed in the sand filter or another part of the water treatment facility but in the raw water source. The benthic layer of the rivers and lake provides a supportive environment for a large nematode population which is suspended in the water column following heavy rains by the scouring action of increased streamflow. Thus a direct relationship exists between nematode density in finished water and rainfall.

Continual sources of nematodes for the rivers and lake, in addition to adjacent agriculture land, are a sewage lagoon and sanitary land fill, both of which flow into the river. Two genera of nematodes which appear in the lagoon effluent also appear in the finished water.

An improved method for the detection of nematodes in water was developed whereby nematodes could be extracted and concentrated onto a 12 mm nucleopore membrane and identified by scanning electron microscopy.

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DETERMINATION OF BREEDING SITES OF NEMATODES IN A MUNICIPAL DRINKING WATER FACILITY

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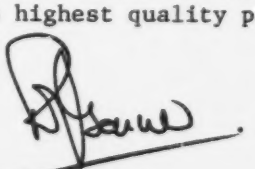
FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The primary mission of the Health Effects Research Laboratory in Cincinnati (HERL) is to provide a sound health effects data base in support of the regulatory activities of the EPA. To this end, HERL conducts a research program to identify, characterize, and quantitate harmful effects of pollutants that may result from exposure to chemical, physical or biological agents found in the environment. In addition to valuable health information generated by these activities, new research techniques and methods are being developed that contribute to a better understanding of human biochemical and physiological functions, and how these functions are altered by low level insults.

This report provides an assessment of the origin and occurrence of nematodes in treated drinking water. The results of this investigation indicated that nematodes could enter treated water if they were present in significant numbers in the source water. No health hazard has been demonstrated from the ingestion of low numbers of these organisms. However, the possibility that nematodes could harbor pathogenic microorganisms if the nematodes originated from heavily polluted environments lends additional support to the premise that water sources used to produce drinking water should be of the highest quality possible.



R. J. Garner
Director
Health Effects Research Laboratory

ABSTRACT

The source of nematodes in finished water has been clearly demonstrated to be in the raw water and not in the sand filter or another part of the water treatment facility. The benthic layer of the rivers and lake provides a supportive environment for a large nematode population which is suspended in the water column by the scouring action of increased streamflow following heavy rains. Thus a direct relationship exists between nematode density in finished water and rainfall.

Continual sources of nematodes for the rivers and lake, in addition to adjacent agriculture land, are a sewage lagoon and sanitary landfill, both of which flow into the river. Three genera of nematodes which appear in the lagoon effluent also appear in the finished water.

An improved method for the detection of nematodes in water was developed whereby nematodes could be extracted and concentrated onto a 12-mm nucleopore membrane and identified by scanning electron microscopy.

This report was submitted in fulfillment of R804292010 by Clemson University under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period February 9, 1976, to August 15, 1977, and work was completed as of August 15, 1977.

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SECTION 1

INTRODUCTION

OBJECTIVES OF STUDY

The overall objective of this study was to determine the source of nematodes that frequently are found in finished potable water. Two hypotheses were that nematodes breed in the sand filter of treatment facilities; and that nematodes enter water treatment facilities in raw surface water and are not completely removed. Both hypotheses were tested at the modern water treatment facility serving Easley, South Carolina.

A secondary objective was to improve the current methodology for extracting, concentrating, and detecting nematodes in drinking water.

SUMMARY OF PREVIOUS WORK

The presence of nematodes in water treatment systems and their potential effects on water quality was reported as early as 1918 when Cobb noted their presence in slow sand filters (1). Cobb's early research has since been followed up by numerous investigators who have shown that nematodes exist in many public water supplies. In the summer of 1953, Kelly observed nematodes in the effluents from slow sand filters of the Norwich, England, water system. Kelly proposed the use of a microstrainer fabric, 23-μm pore size, to remove the nematodes and similar microorganisms (2). During the development of a procedure for detecting the Entameba histolytica cyst in water samples, nematodes were also found in a water supply whose source was the Ohio River (3). This discovery prompted a nationwide survey which eventually confirmed the presence of nematode populations in 16 of 22 public water supplies examined (4).

Questions that need to be addressed concerning nematodes in drinking water are: what are the breeding sites of the nematodes, do they pose a public health problem, and what is the effectiveness of the treatment facility in removing nematodes from raw water.

The sources, or breeding sites, of nematodes have been an obvious point of concern, and Chang and co-workers have discussed two possibilities: first, that the nematodes are of raw water origin (5) or second, that they may be breeding within the treatment plant. It is the ambiguity of this literature more than any other item that has served as the genesis for this particular study.

At present, no problem other than one of aesthetics has been shown to be associated with nematodes in public water supplies. However, some investigators have suggested potential problems more serious in nature, such as disease transmission. Nematodes are among the many invertebrates which serve to decompose sewage in treatment plants. From these plants nematodes are discharged into waterways which may serve as supply sources for public water. Nematodes have been shown to ingest Salmonella and Shigella bacteria and small amounts of Coxsachie and Echo viruses which remained completely protected within the nematode gut even when 90% of the nematodes in the study were immobilized by a 95-100 ppm dosage of chlorine (6). Chang also showed that Salmonella in the nematode gut survived a 10-ppm free chlorine dosage for 15 minutes and remained viable when defecated by the nematode onto a suitable medium; this example indicates that it is possible for a nematode to harbor pathogenic micro-organisms, protect them through chlorine treatment, and then release them to the finished water.

With these observations in mind, we decided to conduct a study which would answer with greater certainty the questions concerning the nematodes' origin.

SECTION 2

CONCLUSIONS

EXTRACTION, CONCENTRATION, AND DETECTION OF NEMATODES IN FINISHED WATER

The procedure developed for this study was field- and laboratory-tested for over a year and has been found to provide data rapidly and with a high degree of reproducibility. This procedure has been compared to the current standard optical microscopic technique and has been shown to be approximately four times as sensitive.

Conclusion

The new nematode collection and detection method is more accurate than the optical technique and has been used extensively during this study.

NEMATODE DENSITY AND ANNUAL CYCLE

After monitoring the nematode density in the finished water of the Easley city plant every two weeks for twelve consecutive months, we found that there was no monthly or annual cycle in the population dynamics. Density varied between 0.5 and 1.5 nematodes per gallon for most of the year, with variations occurring as a result of environmental conditions but not as a consequence of breeding within the sand filters in either the fall or winter.

Conclusion

There is no evidence of a monthly or annual cycle in the population density of nematodes in the finished water.

NEMATODE REMOVAL WITHIN TREATMENT FACILITIES

The search for nematodes in the river and lake showed high densities and similar genera were present in both the raw and finished water. Examination of water samples taken at the beginning, midway through, and at the end of the water treatment process showed that between 90-98% of the nematodes were removed from the water. Two to ten percent of the population passed through to the finished water unharmed as worms.

Conclusion

The water treatment facility removes between 90-98% of the nematodes, leaving between 2-10% in the finished water.

NEMATODE CONCENTRATION IN SAND FILTER

One sand filter was carefully dissected, and samples of water, anthracite, sand, and gravel were removed for examination. A low density of average-sized nematodes was found scattered randomly throughout the filter with no concentration of worms in corners or other relatively undisturbed areas. The density of nematodes at any location in the filter (approximately one per gallon) was not greater than that expected to be present in a similar volume of water passing through the filter.

Conclusion

Nematodes are not breeding within the sand filter.

NEMATODE DENSITY AND RAINFALL

During September and October 1976 a positive correlation was observed to exist between the density of nematodes in both the raw and finished water and the local rainfall, streamflow, and lake turbidity. This relationship was repeated on a small scale following a moderate rainfall in early December and on a larger scale following a major spring rain in April 1977.

Conclusion

Nematodes from the benthos are brought into the water column of a river and lake by the scouring of the river bed following a heavy rain.

NEMATODE DENSITY AND TEMPERATURE

The only time during the 12 months when no nematodes were detected was in late January and early February 1977. This was during an exceptionally cold winter when the water temperature reached new lows and a layer of ice covered much of the lake. At such low temperatures apparently nematode motility is reduced below the level required to keep the animals suspended in the water column. They most likely sink to the bottom, remaining relatively motionless until the water temperature increases, whereupon muscle activity returns, motility is regained, and some nematodes again become distributed throughout the water column. They subsequently enter the water intake pipe with the lake water.

This period of extreme cold provided a rather unique condition in this geographic area in which to test the effects of an uncommonly low water temperature on the population density of nematodes in the finished water.

Conclusion

There is a water temperature below which nematodes apparently lose muscular activity and settle away from the water column; thereby water is free of nematodes.

DENSITY OF NEMATODES IN SEWAGE LAGOON EFFLUENT, RIVER, AND LAKE

Through aerial photography and ground observation a stream was located a quarter mile in length, entering the Saluda River one mile upstream from the treatment plant and originating in the vicinity of a recently closed sanitary landfill. Eight miles upstream is an aerated sewage lagoon with its effluent flowing into the river. Bordering the lake and river are cattle and swine pastures from which runoff flows directly into the body of water. Less than 60 nematodes per gallon were detected entering the river from the sanitary landfill drainage and between 50-1500 nematodes per gallon were detected entering the north arm of the Saluda River from the sewage lagoon.

Conclusion

Organic material apparently enters the river from several sources, thus providing an ideal benthic breeding habitat for nematodes which have entered from waste treatment facilities or from adjacent farm land.

DIVERSITY OF NEMATODES IN SEWAGE EFFLUENT, RIVER, LAKE, AND FINISHED WATER

Among the thirteen genera of nematodes found in sewage effluent entering the river, three (Butlerius, Diplogaster and Rhabditis) comprised 80% of the total. Downstream and below the effluent of the stream draining the sanitary landfill, thirteen genera were again found. However, only five of those entering from the sewage lagoon were present here and only two of the three dominant genera, Diplogaster and Rhabditis, were found. In the lake at the level of the intake pipe, five genera were recovered with no representatives from the thirteen in the lagoon effluent. Finally, in the finished water ten genera were identified; three were also present in the sewage effluent, but Rhabditis was the only sewage dominant genus also recovered from the finished water.

Conclusion

Rhabditis is the only dominant nematode genus entering the river from the sewage lagoon which is also found to be present in finished water.

SUMMARY CONCLUSION

A simple and very fundamental biological principle has been observed concerning the source of nematodes in finished water: as an environment is changed, so will there be a change in organisms and their densities. Biologists know that if a river becomes polluted with organic substances which could serve as nutrients for saprophagous organisms, these organisms will be supported and their population will increase. As the Saluda River has become polluted, the nematode population has increased in the nutrient-rich benthic layer. Following a moderate to heavy rain, when their habitat is disturbed by the scouring action of an increased water flow, the concentration of nematodes in the water column may increase dramatically. The

water treatment facility is capable of removing a high percentage of nematodes, but 2-10% of the worms pass through the final sand filter. If in the reservoir the concentration of the worms increases by an order of magnitude, such as following a moderately heavy rain, so will the number in the finished water be increased by an equal amount. Thus, one approach to removing nematodes from finished water is to correct a fundamental error, that of allowing the high nutrient pollution to flow into source waters.

SECTION 3

RECOMMENDATIONS

1. Our principal recommendation is that the sources of nutrients flowing into rivers and lakes must be controlled so that the growth of nematodes is not encouraged by a continual enrichment of the food supply in their habitat.

2. The filtering efficiency of water treatment facilities and specifically of sand filters relative to the removal of nematodes should be determined for a variety of municipal filters and water qualities.

3. A detailed study should be conducted to determine the composition of organisms, both protozoa and metazoa, in effluent from aerated sewage lagoons and in drainage from sanitary landfills. Theoretically the effluent should be clean, but that is clearly not what we found in this study. To our knowledge there are no data on this particular aspect of effluent from both of these waste disposal systems.

4. Increased consciousness should be exercised by state and federal agencies concerning the proximity of waste treatment effluent to the intake of municipal water supplies.

5. The technique developed for nematode detection should now be evaluated for the extraction, concentration, and identification of waterborne protozoa i.e. Giardia.

6. The possibility that Anonchus, Butlerius, Diplogaster, and Rhabditis nematodes from sewage lagoons could be carriers of bacteria and viruses should be investigated.

SECTION 4

MATERIALS AND METHODS

For this study we chose a municipality which supported a modern water treatment facility, obtained its water from an adjacent reservoir, and showed a consistent nematode population (largely of the family Rhabditidae) in its finished product. The reservoir received water from one major river which had several tributaries. Organic effluent entered the river system from an aerated sewage lagoon and by drainage from a recently closed sanitary landfill. The lagoon was approximately eight miles and the landfill one mile upstream from the reservoir (Figure 1).

Nematode density determinations were made on water samples collected from sewage lagoon effluent, several areas along a river and reservoir, water treatment plant intake, and effluent from settling tanks and sand filters. The basic method developed and used in the 12-month survey of finished water is presented in detail. Modifications to this procedure as required for other water sources are given in the appropriate sections.

SAMPLES FROM FINISHED WATER

Water filtration

All samples were taken within the laboratory of a modern municipal water treatment facility which processes approximately four million gallons of water each day. A series of three eight-inch diameter, two-inch deep, US Standard sieves (Tayler, Inc.) was placed on an eight-inch steel funnel supported by a ring stand near the water tap. The first sieve had a 25-µm pore screen (#500 mesh), followed by two sieves, each with screens of greater pore sizes which served as supports for eight-inch inserts (secured to the frame with opaque bathtub caulk) of polyetheylene woven material (Tetko, Inc.). The second sieve had an insert with 20-µm pores (HD7-20) and the third, an insert with 10-µm pores (HD7-10 Super). Every fourth gallon of water filtered through sieves (25-, 20-, and 10-µm pores) was passed through a standard parabolic, steel filtration funnel with a 47-mm diameter membrane filtration unit, coupled to a 1-gallon vacuum flask. A 47-mm, Unipore^{R} polycarbonate, 3-µm pore membrane (Bio-Rad Laboratories) was placed on the filtration unit through which the sieved water passed. The filtration apparatus for the collection of nematodes is shown in Figure 2. A more detailed description of the procedure follows.

A municipal water meter (Neptune, Inc.) is attached to the sample faucet and adjusted to a flow rate of approximately 1.5 gpm. Water is run through

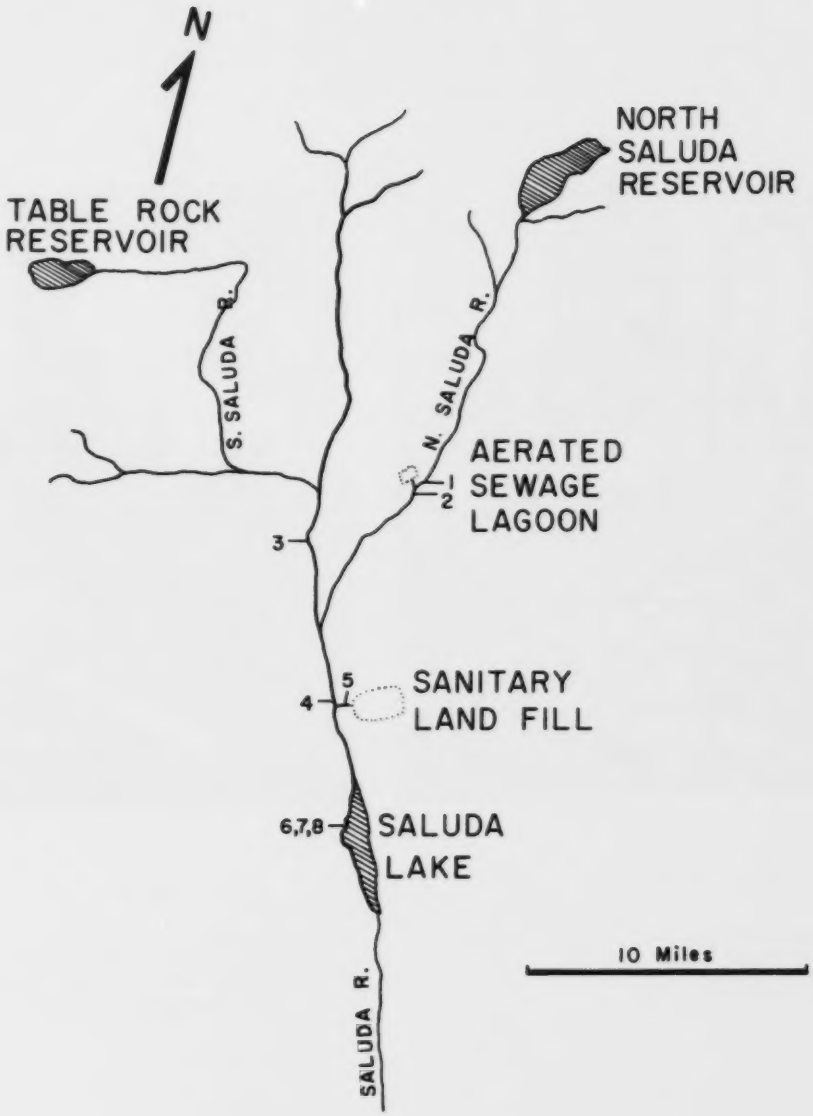

Figure 1

Watershed study area in Easley, South Carolina.

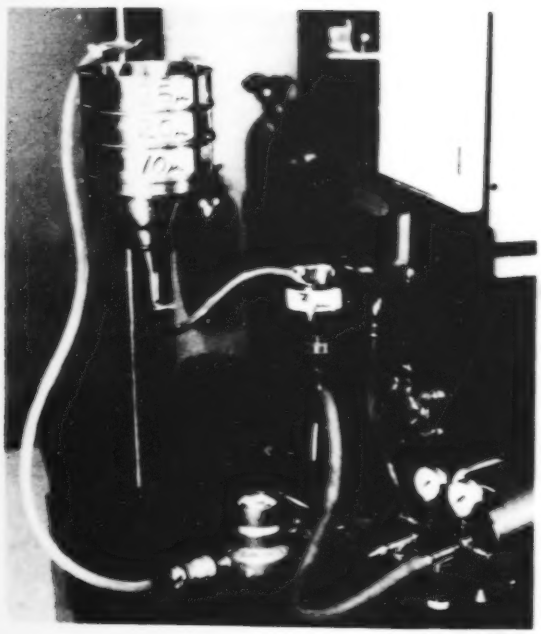

Figure 2

Apparatus for the extraction of particulate matter from drinking water.

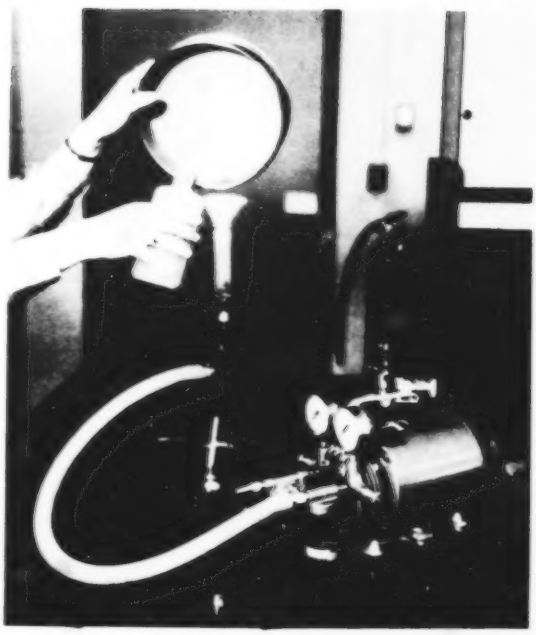

Figure 3

Particulate matter being washed from sieve onto 12-mm membrane in Swinney filter.

the meter and 3/4-inch i.d. plastic tubing for a sufficient period of time to clean the line of rust. The meter reading is recorded, and tubing from the flow meter is placed over the top of the first sieve and secured in that position. At this flow rate no accumulation of water should occur at the 24-µm pore sieve. There is usually a shallow head of water on the second and third sieves, and frequent flow rate adjustments may be necessary to prevent excessive accumulation and overflow. After three gallons have passed through the sieves, the support funnel, and into the sink, the plastic drain hose is quickly moved to the parabolic funnel for filtration through the 47-mm diameter, 3-µm pore polycarbonate membrane. As sieved water begins to fill the funnel, the vacuum pump is turned on to 15 psi to facilitate filtration. When the filtered water approached the one-gallon mark on the vacuum flask, the drain hose is removed from the parabolic funnel and sieved water is directed into the sink. The pump is then turned off, the funnel removed carefully, the flask emptied and the funnel replaced. The sequence of passing one gallon of sieved water through the membrane for each four gallons of sample water filtered through the three sieves is repeated. Ideally, one hundred gallons of water are processed in this manner, 25 of which will have been sieved and also filtered through the membrane.

Optical Microscopy

At this point, one of two methods for the further concentration and identification of nematodes can be employed. The filtered material from each sieve and membrane can be processed wither for optical or scanning electron microscopy. The optical procedure will be discussed first. If there is a small amount of material remaining on each sieve the residue can be transferred to a 13-mm diameter, 3-µm pore membrane in a Swinney (Fisher Scientific) filter; however, if there is a large amount the transfer must be made to a separate 47-mm diameter membrane. The latter is accomplished by repeatedly flushing each tilted sieve with distilled water and collecting the wash in the parabolic funnel with a 47-mm diameter, 3-µm pore membrane. For a small accumulation of material the smaller 13-mm membrane is employed. For this, a one-hole rubber stopper with short tubing inserted is placed onto the top of the vacuum flask. One end of a Swinney filter holder is attached to the plastic tubing and a 20-cc syringe is secured to the other end of the Swinney device. A 3-µm pore polycarbonate membrane is placed within the Swinney and a 3-4 inch diameter plastic funnel is positioned in the neck of the syringe (Figure 3). The transfer is conducted as described above by flushing each of the three sieves and the 47-mm diameter membrane (Figure 4) with distilled water and collecting the material on four separate 13-mm membranes. After the transfer of filtered material to the 3-µm pore membranes is complete, each membrane is placed in a Syracuse watch glass, or similar shallow disk, with a few milliliters of distilled water. The surface of the membrane and the surrounding water are then examined under a stereoscopic microscope for a total nematode count. Selected worms can be removed to a slide, covered with a coverslip, and then identified with bright field or phase optics.

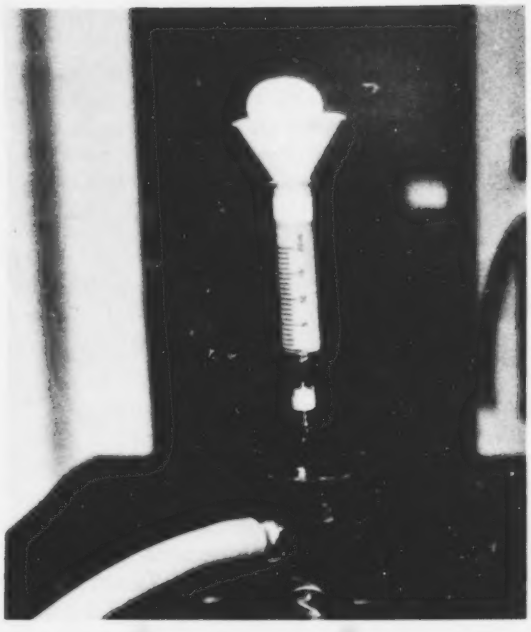

Figure 4

Particulate matter being washed from 47-mm membrane onto 12-mm membrane.

Figure 5

Swinney filter opened and 12-mm membrane being placed onto SEM stub.

Scanning Electron Microscopy

If scanning electron microscopy (SEM) is desired for the detection of nematodes, the residue on each of the three sieves and on the 47-mm membrane must be transferred to 13-mm diameter, 3-μm pore membranes. This is only practical if there is a small accumulation of material on the sieves and membrane. The procedure will be the same as discussed above and the transfer must be carefully and completely made to the smaller membranes (Figures 3 and 4). As the last few milliliters of wash are drawn through the Swinney apparatus, 10-ml of 2% glutaraldehyde are added to the syringe and 3-ml filtered through the Swinney holder. The syringe and Swinney holder are removed together from the short plastic tubing and positioned upright for one hour. This will fix the nematodes so they will not become excessively distorted on air drying. After the nematodes have been in glutaraldehyde for one hour, each syringe is placed back on the plastic tubing and the fixative is replaced with 50% ethanol. The dehydration is continued, allowing each increasing concentration of alcohol (75%, 95%, 100%) to remain in the syringe for 10 minutes. After the second rinse of absolute ethanol has been pulled through the syringe the Swinney holder is removed from the syringe, opened and the membrane removed and placed in a protected area to dry (Figure 5). The particulate matter does not adhere strongly to the membrane and may be lost by any sudden movement of air current. Within ten minutes the membrane is secured to an SEM stub with double stick tape. After metal coating the membrane is examined at approximately 200 X for the detection, identification, counting and sizing of the nematodes. The time required to examine each stub varies from 30-60 minutes, depending on the amount of extraneous material present and the concentration of nematodes. At this magnification, the nematodes are usually identifiable by their characteristic shape (Figure 6). If positive identification is difficult at 200 X, magnification is increased to a power where the cephalic region and cuticular patterns can be seen (Figure 7).

Nematode Densities Calculated

To obtain the total number of nematodes per 100 gallons of sample, from either the optical or scanning electron microscope, the following method is employed:

(a) Number of nematodes present on 25-μm sieve = A
(b) Number of nematodes present on 30-μm sieve = B
(c) Number of nematodes present on 10-μm sieve = C
(d) Number of nematodes present on 3-μm membrane = D

$A + B + C + (4 \times D) = E$ = Number of nematodes/100 gallons

The number of nematodes on the 3-μm membrane is multiplied by a factor of four because only 25 gallons are filtered through the membrane, and a total of 100 gallons is filtered through each sieve. On occasions, we have not been able to process 100 gallons because of excessive inorganic (rust) or organic (algae) material in the drinking water; a fraction of the desired volume, as recorded on the flow meter, will suffice in the calculations.

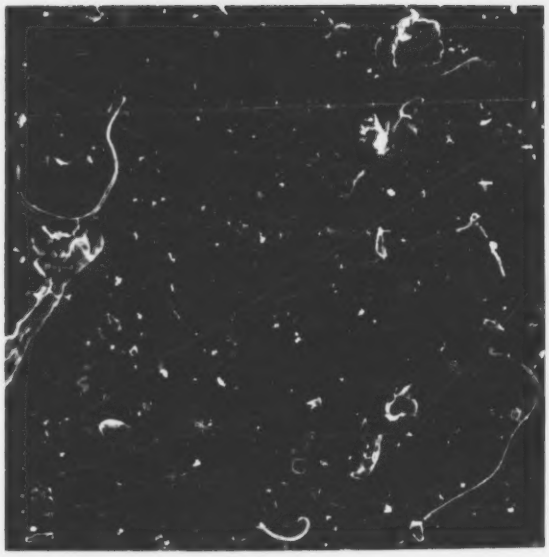

Figure 6

Three nematodes on
3-μm pore membrane in SEM.

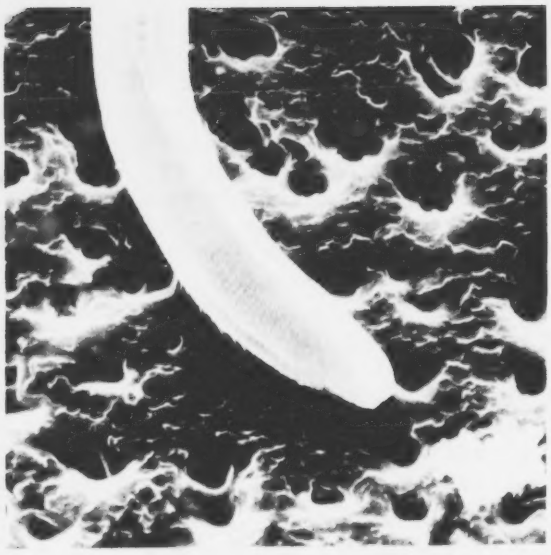

Figure 7

High magnification of anterior
region of nematode showing
annular ridges and mouth.

Comparative Study

To compare the effectiveness of the two procedures and also to test the reproducibility of samples taken in series, the following study was conducted. On alternate days over a ten-day period, two separate samples of drinking water, were taken one hour apart from the water treatment facility laboratory. The first was processed for optical and the second for scanning electron microscopy. Examinations of the collected material were conducted by separate individuals and data were not compared until after five examinations had been completed.

SAMPLES FROM RIVER, LAKE AND SEWAGE LAGOON EFFLUENT

A modification of the centrifugal-floatation technique (7) was used to determine the density of nematodes in water samples taken from eight sites along the Saluda River (Figure 1). On each of six collection dates in May and June of 1977, multiple one-gallon samples were taken from the following locations: 1) fifty feet above the sewage lagoon effluent on the North Saluda, 2) at the mouth of the sewage lagoon effluent, 3) from the South Saluda River, 4) above the effluent from the sanitary landfill, 5) effluent from the landfill, and 6) lake water at the surface, 2 m, and 3 m from the bottom. In the laboratory each one-gallon sample was shaken for one minute and a 320 ml sample was divided equally among eight 50 ml polycarbonate centrifuge tubes. The tubes were balanced and centrifuged at 200 x g for five minutes. The supernatant from each tube was then filtered through a single 47-mm, 3-micra Unipore polycarbonate membrane in a parabella funnel attached to vacuum flask. A stream of distilled water washed material from the membrane into a Syracuse watch glass which was then examined under the dissecting scope.

The residue in each centrifuge tube was resuspended thoroughly in a sugar-water solution (484 g of sugar per liter of distilled water gives a solution with specific gravity of 1.18) and centrifuged as before. The supernatant of each tube was then poured into a 600 ml beaker with 300 ml of tap water, stirred and allowed to settle for about 15 minutes to dilute the syrup and to allow the nematodes to recover from any deleterious osmotic effects of the syrup. The sugar solution centrifugation may be repeated if necessary if the nematode concentration is greater than 100 nematodes/per gallon, as is usually the case with sewage lagoon effluents. After 15 minutes the supernatant and tap water were filtered through a 47-mm, 3-micra pore membrane as above. The material on the membrane was again washed with distilled water into a Syracuse watch glass and examined under a dissecting microscope or transferred to a 13-mm membrane for SEM examination.

SAMPLES FROM SAND FILTER

To ascertain the importance of the sand filter as a nematode-breeding area, random samples of sand, anthracite, and gravel were taken from the filter just before backwashing, and water samples were taken from the backwash effluent. Also the sediment or floc on the bottom of the sedimentation tanks was sampled. Nematodes can be separated and extracted from inert

debris by their motility. Requirements for the technique (8) were a funnel with a piece of rubber tubing attached and closed by a clamp. The funnel was placed on a support and contained the sample on a piece of tissue supported by a wire screen. The funnel was then filled with water until the tissue was submerged. Active nematodes passed through the tissue and collected in the funnel stem. The nematodes were then collected and concentrated in accord with the above procedures for optical or scanning microscopy.

GENERIC DETERMINATION

The procedure used to collect samples at four locations for the determination of generic diversity of nematodes was as follows. The aerated sewage lagoon effluent samples were collected in one-gallon plastic bottles directly from the effluent pipe that empties into the river. The Saluda River samples were collected at a depth of 1/2 m at a point several kilometers below the entrance into the river of the stream draining the sanitary landfill (Figure 1). Lake samples were collected at a depth of 1 m within 2 m of the intake for the water treatment facility. The technique for collecting nematodes from finished water was presented earlier.

Extraction of nematodes from sewage effluent, river, and lake samples was most often accomplished by letting the one-gallon sample of water settle for 24 hours. A 10-ml portion of the bottom layer was then taken with a pipette and placed in a Syracuse watch glass. Two alternate methods were used to a lesser extent. The first involved a steep-sided wine bottle in which the one gallon sample was placed. The neck of the bottle was tightly fitted with an 8 inch rubber hose, with a hose clamp attached to the end to insure water tightness. The bottle was inverted and allowed to stand for 24 hours. A second clamp was placed four inches above the bottom clamp, and a 10-ml sample was collected in a watch glass from the tubing by removing the bottom clamp.

The second method, employed only for river and lake samples, had 25 gallons of water poured through a series of our eight-inch sieves of 500, 106, 53 and 25 micra mesh pores. The trapped sediment on the last two sieves was then washed into a 25-ml sample vial with distilled water and taken to the laboratory for examination in a watch glass.

The liquid in the Syracuse watch glass was examined under a stereo microscope using indirect fluorescent lighting. The nematodes were preserved by both infiltration with glycerin and preservation in 5% formalin. Identification of the nematodes was accomplished under 400 X or 1000 X phase contrast optics with the aid of two taxonomic keys (9, 10).

SECTION 5

RESULTS AND DISCUSSION

EXTRACTION, CONCENTRATION, AND DETECTION OF NEMATODES IN FINISHED WATER

Through the use of the prescribed series of sieves and membranes, an assortment of small plants and animals can be efficiently removed from drinking water. The extracting and concentrating procedures are simple, can be completed within a two-hour period, and utilize equipment costing less than five hundred dollars.

The effectiveness of the SEM method for detection, when compared to the optical procedure, is indicated in Table 1. The SEM was four times more effective, with a total of 167 worms identified, than the stereoscopic microscope with 40 observed from duplicate water samples. The nematodes observed optically from each sieve and membrane were larger than those measured in the SEM. The smallest nematodes recorded were 75μm by 3μm, a measurement which is close to the dimensions of a newly hatched larva, and these were observed only with the SEM. The accuracy of the extraction and concentration procedures when coupled with the SEM for detection is difficult to determine. However, based on limited recovery studies with known numbers of laboratory-reared nematodes of different sizes, we believe that the recovery is approximately 75%. When the 3-μm pore membrane is changed to a 1-μm pore, the rate of water flow is greatly reduced and retention of particulates is increased. When the pore size is increased to 5-μm, fewer nematodes are retained but the rate of water flow is enhanced. Membranes with 3-μm pores were thus judged to be the most desirable.

The effectiveness of these procedures when coupled with stereoscopic microscopy is considerably less than with the SEM. The limited magnification of the optical system, the depth of the water in the Syracuse watch glass, and the arrangement and type of lighting for visualizing the nematodes are all features which limit the detection of the smaller nematodes with optical microscopy.

NEMATODE DENSITIES AND ANNUAL CYCLE

The data in Table 2 present the number of nematodes collected from finished water samples for each collection day, beginning June 18 and ending July 3. It was during this preliminary study that the procedure included only the three eight-inch sieves and not the 3-μm pore membrane. This data, when compared to the subsequent results obtained with the use of the 5-μm membrane, gives an indication of the ability of the nematodes to pass through a 10-μm sieve and also suggests that the majority of the nematodes were motile.

Table 1. A COMPARISON OF THE TWO DETECTION METHODS FOR DETERMINING NEMATODE LENGTH AND POPULATION DENSITY IN DUPLICATE WATER SAMPLES

Filters	Stereoscopic Microscopy		Scanning Electron Microscopy (SEM)	
	Total nematodes from 5 samples (500 gal)	Average nematode length	Total Nematodes from 5 samples (500 gal)	Average nematode length
25 microns	13	485 microns	34	436 microns
20	6	333	20	294
10	4	315	9	305
3	17	405	104	332
Total nematodes	40		167	

Table 2. NEMATODE DENSITY IN FINISHED WATER COLLECTED THROUGH THREE SIEVES, THE SMALLEST WITH A 10-UM PORE SIZE

Date of sample	Nematodes per gallon
6-18-76	0.51
6-21-76	1.67
6-22-76	0.89
6-23-76	0.40
6-25-76	0.14
6-28-76	0.28
7-1-76	0.27
7-3-76	0.18

During the months of July, August, and early September the length of all nematodes collected was determined by measuring each worm on the video screen of the SEM, and that distribution is presented in Table 3. Approximately 70% of all nematodes collected were between 100 and 300 micra in length, indicative of a larval population. Only 15% of the nematodes were less than 100 micra in length. Previous investigators have used dissecting microscopes to confirm the presence of nematodes, and such detection is difficult when the length of the worm is less than 100 micra, especially if the nematode is nonmotile. Identification problems due to size and nonmotility were eliminated with the SEM.

The major period of collection and enumeration extended for 12 months from July 1976. The data in nematodes per gallon of finished water are shown in Figure 8. There is clearly a base line of approximately one per gallon with three sharp deviations: early October, early December, and middle April. These peaks are believed to be a consequence of an environmental perturbation, which will be discussed later, and they are not a consequence of a reproductive cycle which we anticipated finding.

NEMATODE REMOVAL WITHIN TREATMENT FACILITY

By determining the nematode density in (a) raw water entering the treatment plant, (b) the influent to the sand filter, and (c) the finished water, the effectiveness of the treatment process on nematode removel could be evaluated. On each of five collection dates during periods of low rainfall the three samples were taken within a three-hour period and the concentration at each location is expressed in Table 4 as the average of the five samples. For these samples the treatment process removed 98% of the nematodes entering the system. These results are similar to those reported by Gupta (11) who showed that 99% of the nematodes could be removed by coagulation, sedimentation, and filtration, and to Peterson et al. (12) who noted removal of 98% nonmotile and 25% motile nematodes by a similar process.

There was only one sampling date following a period of heavy rain, and the highest concentration of nematodes in the finished water, 15 per gallon, was found on this occasion. Removal efficiency by the treatment processes apparently decreased when the nematode concentration in the water entering the plant was increased because of the heavy rainfall. The entire treatment process removed only 91% of the nematodes (Table 4).

These data also provide evidence that the source of nematodes in the finished water is the raw water and not an area within the treatment plant. Increases in the concentration in the raw water were always accompanied by increases in the influent to the sand filter and in the finished water.

NEMATODE CONCENTRATION IN SAND FILTER

The examination of component parts of the sand filter for nematodes resulted in very few positive samples, approximately one nematode per gallon. If gravid worms had been present in the recesses of the filter where they were protected from the turbulence of back-washing and capable of some sustained production of larvae, both adult and juvenile worms would have

Table 3. LENGTH DISTRIBUTION OF NEMATODES FROM 100-GALLON SAMPLES OF FINISHED WATER COLLECTED ON 12 SEPARATE DATES

Date of Sample	LENGTH DISTRIBUTION (MICRONS)										
	<100	100-199	200-299	300-399	400-499	500-599	600-699	700-799	800-900	900-1000	Total
7-10-76	32	79	18	11	3	0	0	0	0	0	143
7-13-76	24	88	24	9	0	0	0	0	0	0	145
7-19-76	17	58	27	3	2	0	1	0	0	0	108
7-22-76	43	81	23	1	1	1	0	0	0	0	150
7-24-76	12	57	28	15	2	0	0	0	0	0	115
7-25-76	16	37	25	1	0	0	1	0	0	0	80
7-26-76	20	85	41	10	1	0	0	0	0	0	157
8-13-76	4	28	29	6	2	0	0	0	0	0	69
8-18-76	24	31	37	6	16	12	4	0	0	0	130
8-27-76	16	45	25	29	6	5	2	0	0	1	129
9-3-76	1	15	20	11	3	1	0	0	0	0	51
9-14-76	4	16	31	12	4	2	0	1	0	0	70
TOTAL	213	621	328	114	40	21	8	1	0	1	1347

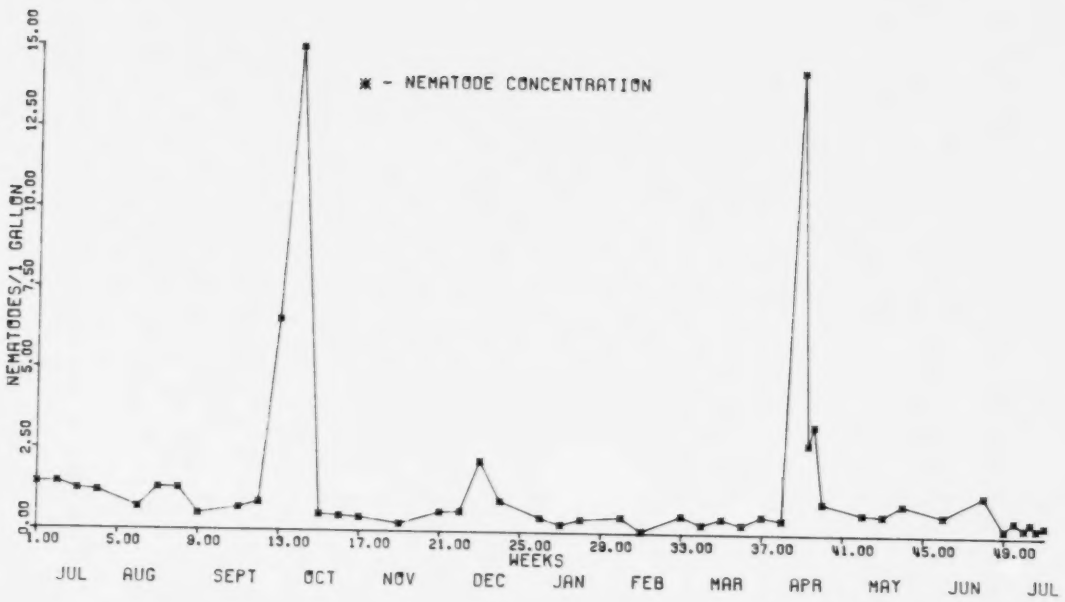

Figure 8

Nematode density in finished water for 54 weeks during 1976-77.

Table 4. NEMATODE CONCENTRATION AT THREE LOCATIONS IN THE TREATMENT PLANT DURING DRY AND RAINY PERIODS

Location	Negligible rainfall		Heavy rainfall	
	Number of nematodes present	Percent reduction of numbers in raw water	Number of nematodes present	Percent reduction of numbers in raw water
Raw water	24		158	
Sand filter influent	3.5	85	64	40
Finished water	0.5	98	15	91

been identified. These few nematodes that were observed were similar in size to those found in the influent to the sand filter and in the finished water.

In the face of these observations, the conclusion of Section B, that the nematodes are not breeding in the sand filter, is supported. It was also determined that the careful and complete sampling of a second filter, with all of the associated technical problems for the treatment plant personnel, would not be necessary.

NEMATODE DENSITY AND RAINFALL

The effects of rainfall were first noted in results of samples collected over the 16-day period by the preliminary procedure utilizing only sieves (Table 2). The first sample of the study contained 0.51 nematodes per gallon. The rainfall for that sample date was measured at 0.66 inches per day. The concentration in the sample two days later increased to 1.67 nematodes per gallon with rainfall recorded at 0.83 inches per day. As rainfall decreased during the following days, nematode concentrations also tended to decrease.

In support of the above observation, increases in nematode concentrations in the finished water after periods of rainfall were also found in samples collected by the finalized procedure (Figure 9). For week 12, during the middle of September, no rainfall was recorded and the concentration of nematodes was 0.86 per gallon. During the following week the concentration increased to 6.53 nematodes per gallon with a rainfall of 0.71 inches per day. The sample for week 13 resulted in a concentration of 15.0 nematodes per gallon with an accompanying rainfall of 2.17 inches per day. The next week no rainfall was recorded, and the concentration dropped to a low 0.51 per gallon.

Rainfall may increase the concentration of nematodes in the finished water in two ways. First, Englebrech, et al., noted significant increases in the nematode population of a stream during periods of high runoff (13). Nematodes are known to be abundant in the top few inches of soil; consequently, rainfall with intensities large enough to cause runoff probably flushes the nematodes from the soil into a receiving stream. These nematodes are then carried by the stream to the intake of the water treatment facility and eventually are observable in the finished water. The land in the area of Saluda River and Saluda Lake is hilly and used for farming. Although we did not conduct surveys, nematodes in fields and pastures should be abundant and are probably flushed into the river by heavy rainfall. The increase in nematode concentrations in the finished water for week 14 may partially be the result of runoff. Prior to this sample which resulted in a total of 15 nematodes per gallon, the highest intensity of rainfall was recorded. At one rainfall station, 4.78 inches of rain fell in a 24-hour period. Such information suggests that runoff very probably contributes to the nematode population in the raw water and also to the concentration in the finished water.

Secondly, Baliga et al., found that the concentration of nematodes in the benthos of a stream decreased following high flows in the stream (7).

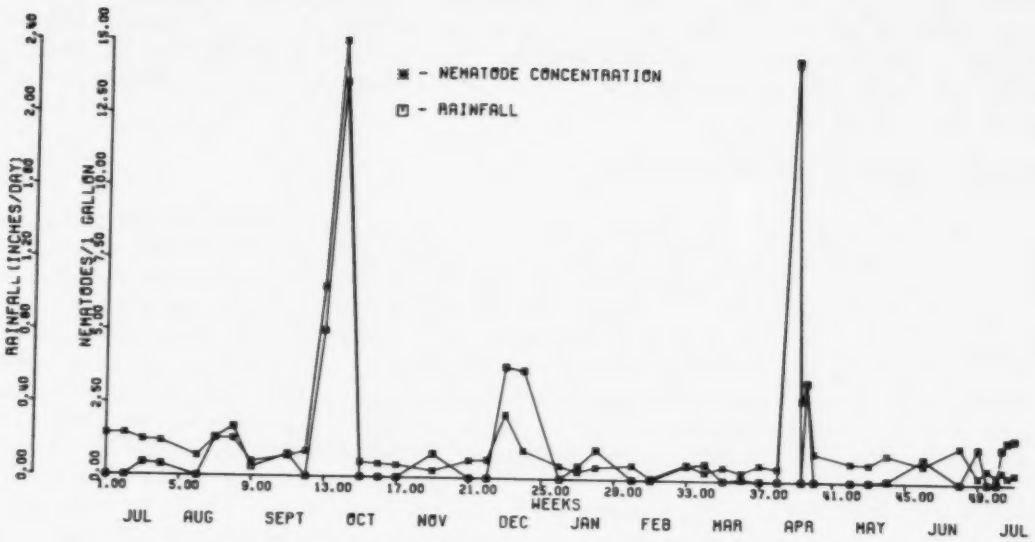

Figure 9

Nematode density in finished water and the average rainfall in the watershed area for 54 weeks during 1976-77.

The drop in density, they concluded, was as a consequence of scouring the nematodes from the benthic area. In our study, high concentrations of nematodes were found in the finished water during periods of increased streamflow of the Saluda River. Such increased water flow very probably moved the nematodes from the benthos into the water column and carried them to the lake and subsequently to the intake pipe of the water treatment plant. Streamflow measurements were obtained from a United States Geologic Survey (U.S.G.S.) station located about 1.2 miles downstream from the water plant. The relationship between stream flow and nematode concentrations in the finished water is shown in Figure 10.

Incidental to this rather strong relationship between the forces associated with streamflow and the movement of nematodes from the benthic area into the water column is the change in turbidity of the river. Thus, a direct correlation exists between the lake turbidity monitored at the water treatment facility and density of nematodes in finished water, as is shown in Figure 11.

NEMATODE DENSITY AND TEMPERATURE

Previous investigations (7) have indicated that nematode concentrations in a stream benthos are higher during the cooler months and lower during warmer months. Similar observations were recorded in this study which indicates that nematode concentration in the finished water generally decreases with decreasing water temperature. When density data from July to January, except for samples during rainy periods, are plotted against water temperature a relationship between nematodes in the finished water and the temperature of the finished water is detected and is presented in Figure 12. The concentrations in water are highest at a temperature of 23-26°C and the lowest at a temperature of 4°C. Motility decreases as the temperature decreases and the less motile worms are removed more effectively by filtration (11). This results in a decrease in the nematode concentration of the finished water.

This relationship is greatly strengthened by the observation that no nematodes could be detected in the finished water during the period in January when the water temperature went below 4°C and ice was on the lake.

DENSITY OF NEMATODES IN SEWAGE LAGOON EFFLUENT, RIVER AND LAKE

There is an increase in the density of nematodes as the river flows toward the lake (Table 5). The South Saluda does not receive the agricultural, industrual, or municipal drainage that flows into the North Saluda and this is apparent in a lower average nematode count. The density of worms from the lagoon effluent is artificially low after the third sample collection because an increased level of chlorine is added to the effluent. The presence of chlorine gas was noticeably greater after a conversation with the plant operator when the high nematode density in the previous collection was mentioned.

After the North and South Saluda rivers merge the average density just above the landfill effluent was 18 nematodes per gallon. Twelve per gallon

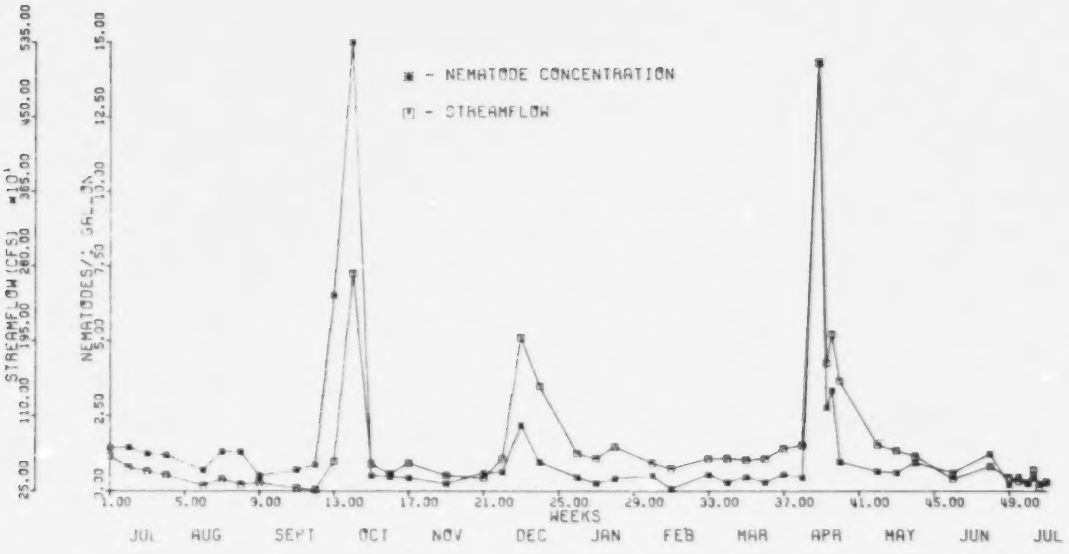

Figure 10

Nematode density in finished water and the streamflow for 54 weeks during 1976-77.

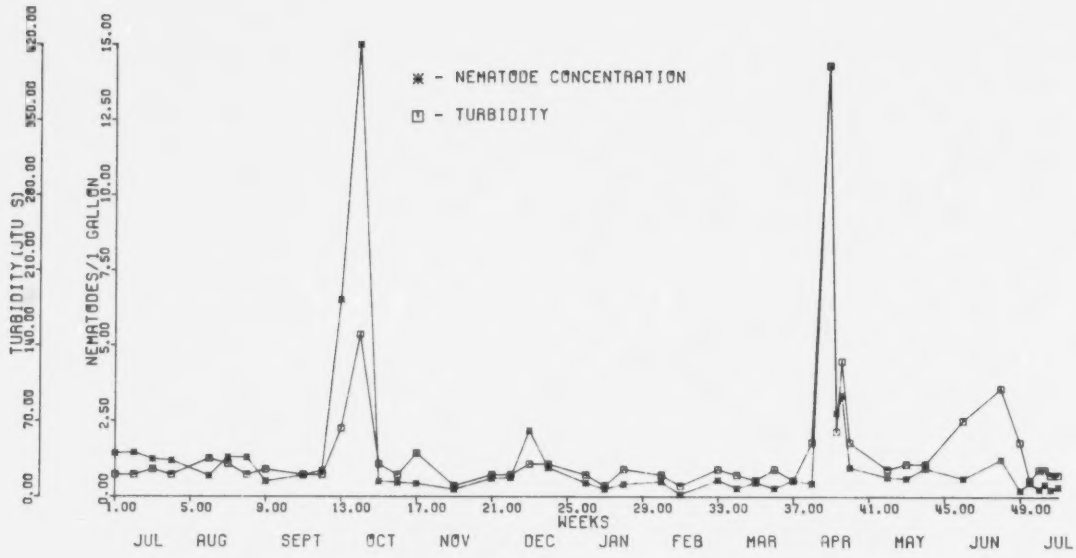

Figure 11

Nematode density in finished water and the turbidity of the lake water for 54 weeks during 1976-77.

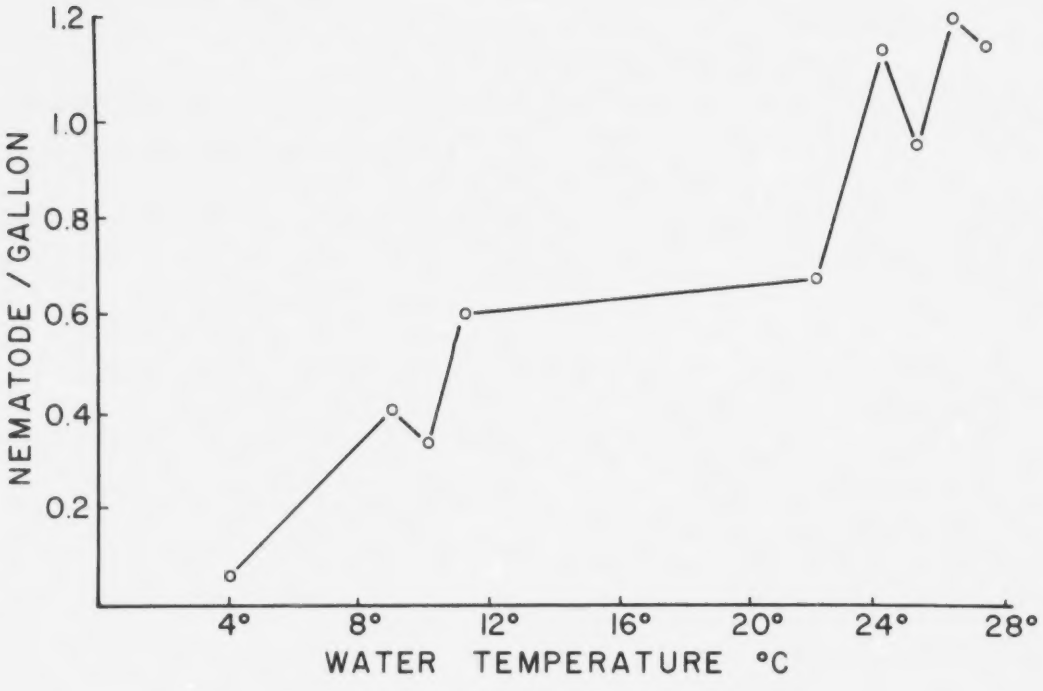

Figure 12

Relationship between nematode concentration in finished water and water temperature.

Table 5. DENSITY OF NEMATODES AT VARIOUS SITES ALONG THE SALUDA RIVER DURING MAY AND JUNE 1977

	Number of nematodes per gallon						
Location	5-6	5-11	5-20	6-2	6-17	6-27	Average
South Saluda River	0	12	0	0	12	0	4
North Saluda River	0	12	12	24	12	0	10
Sewage lagoon effluent	378	1,488	473	71	159	59	438
Saluda River	35	12	12	0	12	35	18
Sanitary landfill effluent	35	24	35	24	0	59	30
Lake Saluda, surface	12	12	12	0	0	0	6
Lake Saluda, 2 m from bottom	35	35	0	0	0	24	16
Lake Saluda, 1 m from bottom	24	12	35	0	0	35	18

is the mean average for the samples from the entire river system if the two effluent figures are not counted. In several samples, and especially on May 11, the nematode population in the Saluda River was the same as that in either of its forks before the sewage effluent entered the river. The sewage effluent, then, appeared to be effectively diluted by the river drainage system. Along the eight-mile course downstream the nematodes were probably well dispersed through the water or eventually settled out, especially during dry periods with no runoff and low flow rate.

The same relationship was found to be true of the effluent from the sanitary landfill. Although the concentration of nematodes in the drainage stream was slightly higher than that in the river, by the time the water reached the lake the density had dropped to its previous level.

The density of nematodes in the lake was stratified with increasing numbers toward the bottom; however, no benthic samples were taken. The intake to the water treatment plant is located about 1.5 m from the bottom of the lake, and it is near that level that the greatest concentration of nematodes was found in the water column.

The summer months of June and July 1977 were extremely dry. With no rainfall and with a resulting minimal streamflow the population density of the river was very low, and in some cases no nematodes were found.

DIVERSITY OF NEMATODES IN SEWAGE EFFLUENT, RIVER, LAKE AND FINISHED WATER

The nematode genera and the frequency of their appearance in samples from four collection sites are given in Table 6. The water samples were taken and generic determinations made during the summer of 1977. Eighty percent of the nematodes in the lagoon effluent represented three genera: Butlerius, Diplogaster and Rhabditis; ten genera composed the remaining twenty percent. The three major genera have been associated by other authors with waste treatment ecosystems (1, 7, 14, 15).

The river samples provided a wide variety of nematodes, thirteen genera, but without one genus or a group of genera dominating. Of the three major genera in the sewage effluent Butlerius was the only one not found to be present in the river. Of the thirteen genera in the river five were observed in the sewage effluent: Anonchus, Diplogaster, Monhystrella, Rhabditis and Tobrilus.

The Lake Saluda sample, taken at a depth of 1 m but 2 m above the intake to the water treatment facility, contained only five genera in a sample of ten nematodes. Dorylaimoides was the predominant genus. It is most interesting that none of the five had been observed in the sewage effluent or in the river sample and none appear on the list of nematodes from finished water.

In finished water seventeen nematodes were identified and were found to represent ten genera. Three genera, Anonchus, Nothotytenchus and Rhabditis were on the list of genera in the sewage lagoon effluent, and together they

Table 6. GENERA OF NEMATODES AND FREQUENCY OF THEIR OBSERVATIONS FROM FOUR LOCATIONS ALONG THE SALUDA RIVER BASIN

Sewage lagoon effluent		Saluda River		Lake Saluda		Finished Water	
Acrobeloides	1	Actinolaimus	1	Dorylaimoides	1	Anaplectus	1
Allionema	1	Alaimus	1	Monochromadora	2	Anonchus	1
Anonchus	1	Anonchus	1	Mononchus	2	Leptolaimus	2
Butlerius	23	Desmolaimus	1	Oncholaimus	1	Leptonchus	1
Cephalobus	1	Diplogaster	1	Prochromadorella	1	Microlaimus	1
Diplogaster	12	Domorganus	1			Mylonchulus	2
Diploscapter	1	Monhystrella	2			Nothotytenchus	5
Monhystrella	2	Paractinolaimus	1			Prodesmodora	1
Mononchoides	3	Rhabditis	2			Rhabditis	1
Nothotyteuchus	1	Rhabdolaimus	1			Rhabdolaimus	2
Plectus	1	Teratocephalus	2				
Rhabditis	31	Tobrilus	1				
Tobrilus	4	Tylencholaimus	1				

represent 41% of the finished water nematode population. Of those three, Anonchus and Rhabditis were also present in the Saluda River.

Therefore, of the thirteen genera in the sewage lagoon effluent, five were identified in the river approximately six miles downstream and two (Anonchus and Rhabditis) of these five were identified in the finished water. It is interesting that none of the five genera identified from Lake Saluda appeared at any other area examined.

Even though the same nematode genera were found in finished water as are known to enter the river-reservoir system from a sewage lagoon eight miles upstream, it certainly does not provide conclusive evidence that the nematodes are from the same source. It merely suggests that they may be from the same source. Attempts to tag large numbers of nematodes entering the river from the lagoon with a fluorescent marker and recapturing the nematodes at points downstream were begun, but technical problems were not solved before the project was terminated.

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